Proc. Natl. Acad. Sci. USA
Vol. 74, No. 4, pp. 1308–1309, April 1977
Chemistry

Nitrogen-15 nuclear magnetic resonance of aliphatic tripeptides

(peptide sequencing/^{15}N chemical shift/NH_2-terminal amino acid/COOH-terminal amino acid/amino acid nitrogens)

VOLKER MARKOWSKI, TAMAR B. POSNER, PHILIP LOFTUS, AND JOHN D. ROBERTS

Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, California 91125

Contributed by John D. Roberts, January 7, 1977

ABSTRACT **The ^{15}N chemical shifts of eight aliphatic tripeptides have been measured at the natural-abundance level. For a given tripeptide, the resonances of the COOH-terminal and NH_2-terminal amino acids can be identified by measurements at low or high pH. The shifts of the NH_2-terminal amino acid nitrogens are essentially independent of the amino acids in the rest of the peptide. The shifts of the other nitrogens are characteristic of the amino acids themselves and of the immediately preceding amino acid toward the NH_2 terminus. Nonterminal amide nitrogens have shifts of about 6 ppm upfield of COOH-terminal amide nitrogens at the isoelectric point of measurement. ^{15}N chemical shifts appear to have considerable potential value for peptide sequencing.**

Although carbon-13 nuclear magnetic resonance spectra can be used to distinguish between NH_2-terminal, COOH-terminal, and nonterminal residues in small peptides (1, 2), the observed shifts are independent of the nature of the adjacent amino acid residue (3). For this reason, ^{13}C chemical shifts are unable to provide much sequencing information for peptides containing more than three residues. In a recent communication (4), we have shown that resonance signals of nitrogen-15 are not subject to this limitation and show a distinct neighboring residue-dependence for simple aliphatic dipeptides. The individuality of these shifts indicates that ^{15}N nuclear magnetic resonance spectra might play a useful role as a nondestructive method for sequence analysis of peptides and, except for sensitivity problems, should be superior to ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. We provide here a further test of this expectation through studies of the ^{15}N nuclear magnetic resonance spectra of some aliphatic tripeptides and also of dipeptides containing more complex amino acid residues than investigated earlier.

EXPERIMENTAL SECTION

All of the peptides were commercially available materials and were used without further purification. The spectra were taken of 0.1–0.2 M peptide solutions in water, and unless otherwise noted, the pH values were those of the isoelectric points. The spectra were recorded at 18.25 MHz on a Bruker WH-180 pulse spectrometer described in detail elsewhere (5). Reasonable signal-to-noise ratios could be obtained over 2–6 hr with the aid of proton noise decoupling, a 30 μsec pulse width (30° flip angle), and a repetition rate of 2 sec. The chemical shifts reported are in ppm upfield from 1 M external $D^{15}NO_3$, which also was used as the external field-frequency lock.

RESULTS AND DISCUSSION

The ^{15}N chemical shifts of eight tripeptides are summarized in Table 1, and for comparison, some of the shifts previously reported for dipeptides are given in Table 2. For Tables 1 and 2, it will be seen that, although the chemical shifts of the nitrogens of the NH_2-terminal amino acids of peptides range over nearly 15 ppm, within a few tenths of a ppm, they are the same as for the corresponding dipeptide, irrespective of the other amino acids in the chain. Clearly, the magnetic environment of the ammonium group in tripeptides shows only small changes when the other amino acids are varied, and this is to be expected if the peptide bonds are in a trans configuration. The agreement between the two data sets is impressive, especially when the measurements were often made at somewhat different pH values in the vicinity of the isoelectric point. The ammonium nitrogen shifts are insensitive to pH changes as long as the amine nitrogen exists in the protonated form, as Table 3 shows. Deprotonation of the ammonium group results in an upfield shift of 13 ppm, as can be seen for triglycine. Any question as to whether a high-field ^{15}N resonance arises from an ammonium group can be answered by raising the pH, which only results in a substantial shift change for the resonance of the NH_2-terminal group.

The consistency of the NH_2-terminal nitrogen shifts indicates considerable utility for determining the identity of the NH_2-terminal residue, at least of peptides made up of aliphatic amino acids. It is possible that the presence of amino acids with more complex side chains might result in deviations from this simple behavior and, although an extensive investigation was not made of this possibility, we have studied several peptides containing aromatic side chains. The results are shown in Table 4, and it will be seen that the ammonium nitrogen shifts in Phe-Ala and Phe-Leu are very similar (346.9 and 346.8 ppm, respectively). The solubility of Phe-Gly was low and this sample was run in acid solution, but it is interesting to note that triglycine shifts 12.9 ppm to low field on going from pH 9 to pH 1.5, while Phe-Gly at 4.9 is shifted 9.5 ppm to low field when compared to Phe-Ala at pH 9. The ammonium nitrogen shifts of 346.2 ppm at pH 12 for Phe-Gly-Gly, very close to the values obtained for Phe-Ala and Phe-Leu shows that the difference arises from the influence of pH. We conclude from these results that phenylalanine also has a distinctive NH_2-terminal shift.

The possibility that nonaliphatic side chains in a peptide might perturb the shifts of adjacent amino acids was also in-

Table 1. ^{15}N Chemical shifts of aliphatic tripeptides (in ppm upfield from $D^{15}NO_3$)

Peptide	Ammonium-NH_2	Nonterminal-NH_2	COOH-terminal-NH
Gly-Gly-Gly	348.9	266.7	260.7
Ala-Gly-Gly	334.7	266.6	260.6
Leu-Gly-Gly	336.4	263.7	260.5
Val-Gly-Gly	340.1	262.3	260.3
Gly-Gly-Ala	348.9	266.6	246.5
Ala-Ala-Ala	335.4	251.8	247.0
Gly-Ala-Gly	349.6	252.2	261.7
Gly-Leu-Gly	349.1	253.8	259.9

Table 2. ^{15}N Chemical shifts of aliphatic dipeptides (in ppm upfield from $D^{15}NO_3$)

Peptide	Ammonium-NH_2	Amide-NH_2
Gly-Gly	348.9	260.4
Ala-Gly	335.0	260.4
Leu-Gly	336.8	257.1
Val-Gly	340.2	255.8
Gly-Ala	348.4	245.9
Gly-Leu	348.9	248.1
Ala-Ala	335.4	246.1

vestigated, and it was found that Gly-Phe and Gly-Tyr give NH_2-terminal nitrogen shifts of 349.1 and 349.0 ppm, respectively. These shifts are very close to those for Gly-Gly of 348.9 ppm. Consequently, it would appear that the presence of the aromatic residue has a negligible effect, at least on the neighboring glycyl residue.

The results in Tables 1 and 2 show that the COOH-terminal nitrogen shifts in the tripeptides are essentially identical to the COOH-terminal amide nitrogen shifts in the corresponding dipeptides. These shifts, and the fact that deprotonation of the ammonium group does not change the amide nitrogen resonance of diglycine (6), indicate that changes at the nitrogen in the adjacent amino acid have no effect on the following amide nitrogen. Comparison of the nonterminal amide nitrogen shifts with those of the corresponding dipeptides also shows that the nonterminal shift is consistently about 6 ppm to higher field than that of the appropriate COOH-terminal residue. This 6-ppm nitrogen shift difference between nonterminal and COOH-terminal amino acid at the isoelectric point is clearly the result of the electrical effect of the $CO_2^{\ominus}$ group because, as can be seen from Table 3, at low pH values these kinds of nitrogens have nearly equal shifts in triglycine. It is important to note that, as with dipeptides, the chemical shift of the nonterminal amino resonance is only dependent on the nature of the amino acid and the nature of the preceding amino acid.

The amide nitrogen resonances of tripeptides range over approximately 20 ppm. Determination of whether a particular resonance arises from a COOH- or nonterminal unit is easily made by lowering the pH, as this has no effect on the nonterminal amide resonance while it shifts the COOH-terminal amino acid 6 ppm to lower field (Table 3).

Table 1 shows that change of a NH_2-terminal glycyl group to an alanyl group has no effect on the nonterminal amide resonance, and this was also observed for dipeptides (4). The change from NH_2-terminal glycyl to NH_2-terminal leucyl or valyl causes the nonterminal glycyl amide resonance to move downfield by 3.0 and 4.4 ppm, respectively. The corresponding downfield shifts caused by NH_2-terminal leucyl and valyl average 2.3 and 3.9 ppm, respectively, for aliphatic dipeptides. The magnitude of these shifts seems somewhat greater for the tripeptides, but the order is the same, with valine producing the largest downfield shift.

Table 3. Dependence of ^{15}N chemical shifts of triglycine on pH (in ppm upfield from $D^{15}NO_3$)

pH	Amino-NH_2	Nonterminal-NH_2	COOH-terminal-NH
1.5	348.8	266.4	267.2
11.9	361.7	266.8	260.6

Table 4. ^{15}N chemical shifts for some dipeptides containing aromatic amino acids (in ppm upfield from $D^{15}NO_3$)

Dipeptide	pH	Ammonium-NH_2	Amide-NH_2
Phe-Gly	4.9	337.4	257.3
Phe-Ala	9	346.9	250.3
Phe-Leu	12	346.8	246.6
Gly-Phe	9	349.1	250.7
Gly-Tyr	11	349.0	250.6

Assuming we know the overall amino acid composition of a tripeptide and the ^{15}N shifts at different pH values, we can determine the sequence from the characteristic ^{15}N shift of the NH_2-terminal amino acid (which is essentially independent of the rest of the sequence), and by comparison of the dipeptides containing the other amino acids. Thus, if 349, 260, and 254 ppm were observed, the pH changes might show that 349 ppm was the NH_2-terminal and 260 ppm was the COOH-terminal amino acid. If the composition is Gly_2-Leu, we can be reasonably confident that glycine is NH_2-terminal. The Leu-Gly dipeptide has its COOH-terminal resonance at 257.1 and Gly-Leu at 248.1 ppm. The best fit for the sequence is clearly Gly-Leu-Gly, and we could check this with the shift for the nonterminal amino acid resonance of 254 ppm.

The important conclusion, at least for the amino acids studied here, is that the ^{15}N chemical shift of a given amino acid residue would appear to depend on whether it is NH_2-terminal, COOH-terminal, or nonterminal, and on the nature of the adjacent residue toward the NH_2-terminus of the peptide.

We conclude that ^{15}N nuclear magnetic resonance spectroscopy could provide a useful method for the nondestructive determination of the amino acid sequence in di- and tripeptides and may also be valuable for more complex peptides. An advantage of ^{15}N over ^{1}H or ^{13}C nuclear magnetic resonance spectroscopy for this purpose is that, with proton noise decoupling, only a single resonance line is expected for each aliphatic amino acid in the chain, provided there is exclusively a trans configuration or a cis configuration at each peptide bond. If multiple resonances were observed, this would be evidence for mixtures of cis and trans amino isomers (7).

This is contribution no. 5490 from the Gates and Crellin Laboratories of Chemistry. These studies were supported by the U.S. Public Health Service Grant GM-11072, from the Division of General Medical Sciences, and by the National Science Foundation.

1. Deslauriers, R. E. & Smith, I. C. (1975) in *Topics in Carbon-13 NMR Spectroscopy*, ed. Levy, G. C. (John Wiley, Interscience, New York), Vol. 2, 2–66.
2. Keim, P., Vigna, R. A., Nigen, A. M., Morrow, J. S. & Gurd, F. R. N. (1974) *J. Biol. Chem.* **249**, 4149–4156.
3. Christl, M. & Roberts, J. D. (1972) *J. Am. Chem. Soc.* **94**, 4565–4573.
4. Posner, T. B., Markowski, V., Loftus, P. & Roberts, J. D. (1975) *Chem. Commun.* **1975**, 769–770.
5. Gust, D., Moon, R. B. & Roberts, J. D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4696–4700.
6. Irving, C. S. & Lapidot, A. (1975) *J. Am. Chem. Soc.* **97**, 5945–5946.
7. Williamson, K. L. & Roberts, J. D. (1976) *J. Am. Chem. Soc.* **98**, 5082–5086.